

Claims

What is claimed is:

1. A recombinant single-chain tri-specific antibody (scTsAb) constructed by fusing anti-tumor associated antigen (TAA) antibody, interlinker, anti-CD3 antibody, interlinker and anti-CD28 antibody in turn.
2. The scTsAb of claim 1 wherein the said anti-TAA antibody, anti-CD3 antibody and anti-CD28 antibody are single chain fragment of variable region (scFv), Fab fragment or single-domain antibody.
3. The scTsAb of claim 1 wherein C myc tag and histidine tag are added alternatively or simultaneously at its C terminal.
4. The scTsAb of claim 1 wherein the said TAA is CarcinoEmbryonic Antigen (CEA).
5. The scTsAb of claim 1 wherein it is fused by anti-CEA single chain fragment of variable region(scFv), FC interlinker, anti-CD3 scFv, HSA interlinker and anti-CD28 single-domain antibody in tandem, comprising the amino acid residues sequence of SEQ ID NO:4.
6. An encoded DNA sequence for scTsAbs of claim 1-5.
7. The encoded DNA sequence for the scTsAb of claim 5, comprising the nucleic acid sequence of SEQ ID NO:3.
8. The scTsAb of claim 5 wherein anti-CEA scFv comprises the amino acid residues sequence of SEQ ID NO:1.
9. The scTsAb of claim 5 wherein anti-CD3 scFv comprises the amino acid residues sequence of SEQ ID NO:2.
10. An expression vector comprising the DNA sequence of claim 6 and claim 7.
11. The expression vector of claim 10 wherein it is CEA-scTsAb/pTRI.
12. A host cell comprising the expression vector of claim 10.
13. The host cell of claim 12 wherein it is Escherichia coli.
14. A method for promoting cytoplasmic soluble expression of scTsAb by inducing exponentially growing host cells containing the coding DNA fragment for scTsAb to express with IPTG at 30°C, wherein the concentration of IPTG is 0.4mmol/ml.
15. The method of claim 14 wherein the host cell is Escherichia coli strain BL21(DE3)

star comprising CEA-scTsAb/pTRI of claim 11.

16. A method for purifying the scTsAb of claim 14 with DEAE anion exchange chromatography.
17. A drug combination for treating or preventing cancer comprising the scTsAb of claim 1-5.
18. The drug combination of claim 17 wherein the cancer expresses CEA
19. An application for treating or preventing cancer with scTsAb of claim 1-5 in.
20. The application of claim 19 wherein the cancer expresses CEA.